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(54) METHOD FOR ANALYZING SEQUENCE OF PEPTIDE AMINO ACID

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a method for efficiently analyzing a peptide amino acid, and more particularly a method whereby an amino acid sequence of even a peptide which cannot be formed to fragment ions by the conventional mass spectrometry can be analyzed.

SOLUTION: According to this method for analyzing the peptide amino acid sequence, the peptide is labeled with a fluorescent compound, and the obtained fluorescent-labeled peptide is analyzed by the MALDI-TOF mass spectrometry using the PSD method.

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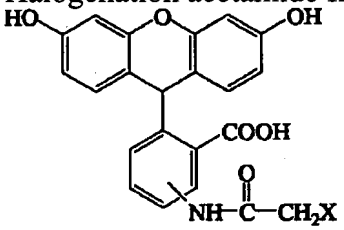
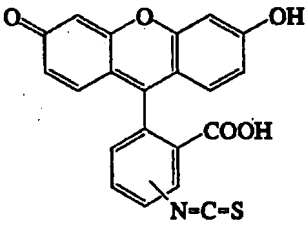
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the analysis approach of the amino-acid-residue array of a peptide.
[0002]
[Description of the Prior Art] It is important to determine the amino-acid-residue array of a peptide or protein, also in order to solve the variegated function of the protein which is the major component of a life object. Effectiveness was very bad although amino-acid-residue array analysis of a peptide or the peptide which fragmented the protein of a giant molecule with the enzyme etc. had been conventionally performed with the chemical approaches, such as an Edman degradation method, or other biochemical processes. In recent years, array analysis of a peptide or small molecule protein came to be performed using the mass spectrometry. It is an advantage that it can analyze by the peptide sample of a minute amount, and this approach is very useful, also when the amino acid of a peptide is permuted or it is embellished. the peptide array analysis which used the Post-Sourse Decay (PSD) technique of the MALDI-TOF-MS method (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry, matrix exchange laser absorption ionization time-of-flight-mass-spectrometry machine) also in some technique of a mass spectrometry recently -- ** -- it is increasingly used as a leading means.
[0003] However, in a certain kind of a peptide or small protein, even if it measures by this MALDI-TOF-MS-PSD method, the ion of an amino-acid-residue fragment is not detected, therefore array analysis of such a peptide or small protein cannot be performed. Now, the cause that this technical point of the TOF-MS-PDS method is difficult is not solved. moreover, above-mentioned MALDI-TOF-MS-PSD in the array analysis of a peptide -- in law, since it often happens that it is difficult in a certain kind of a peptide and small protein to use a sample as fragment ion by excitation by laser and the mechanism of that not fragmenting is not solved, it is not easy to improve a MALDI-TOF-MS spectroscope and to conquer this difficult trouble at present. It is required to devise so that the peptide of a sample and the small protein may be excited by laser light and fragment ion may tend to come out from this, in order to solve these troubles.
[0004] As the technique of this direction, it is a mass spectrometry instead of the MALDI-TOF-MS technique. The method of determining an amino acid sequence by the peptide sample which fixed the charge to dimethyl alkyl ammonium salt for the amino terminal of a peptide is tried using the ChemicallyInduced Detection (CID) method [an international journal mass gamma-ray spectrometry, the 100th volume (Int.J.Mass Spectrometry, Ion Process) of an ion process, and the 287-299th page (1988)]. this technique -- the CID method -- it is -- TOF-MS-PSD -- there is a trouble that measurement is difficult (it is hard to ionize), and cannot say it as a general approach rather than law.
[0005]
[Problem(s) to be Solved by the Invention] This invention solves the above-mentioned trouble and aims at offering the efficient amino acid analysis approach of a peptide. By the conventional mass

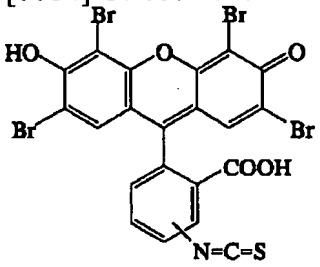
spectrometry, the peptide which cannot carry out [fragment ion]-izing is also aimed more at offering how an amino acid sequence is analyzable in a detail.
[0006]
[Means for Solving the Problem] this invention persons came to complete header this invention for the ability of the amino-acid-residue array of a peptide to be determined by adding a specific fluorescent material to a peptide and analyzing the MALDI-TOF-MAS-PSD spectrum, as a result of inquiring wholeheartedly that the above-mentioned technical problem should be solved.
[0007] That is, this invention includes the following invention.
(1) The amino acid sequence analysis approach of the peptide characterized by being a fluorescence compound, carrying out the indicator of the peptide, and analyzing the obtained fluorescence labeling peptide by the MALDI-TOF mass analysis using the PSD method.
(2) The approach of the aforementioned (1) publication that a fluorescence compound is a fluorescein compound.
[0008]
[Embodiment of the Invention] Hereafter, this invention is explained concretely. The "fluorescence compound" as used in this specification means a compound with the capacity to give off fluorescence. Moreover, the "peptide" as used in this specification means the compound which two or more amino acid combines by peptide linkage, and produces.
[0009] Although especially the peptide used as a sample in this invention is not limited, its with a molecular weight of 5000 or less peptide is desirable. In analyzing the protein which consists of two or more peptide chains (polymer), or the peptide with which a disulfide bond exists, it analyzes, after decomposing into one peptide chain which does not have a disulfide bond by the approach usually used, respectively.
[0010] Although the nucleus peptide (CDYEGRLI) which intracellular protein disassembled as a peptide sample is used for an example and here explains it, according to the approach of indicating below, this invention can be carried out also about other peptides. the MALDI-TOF-MS spectroscope with which this peptide is conventionally used so that the mass spectrum of CDYEGRLI by the MALDI-TOF-MS spectroscope (PSD law) shown in drawing 1 may also show -- PSD -- even if it measures using law, fragment ion cannot be detected, but in a conventional method, an amino-acid-residue array is the peptide which cannot be determined.
[0011] In this invention, before measuring a peptide with a mass spectrograph, the indicator of the peptide concerned is carried out with a fluorescence compound. Although it will not be limited especially if it is generally used by this technical field as a fluorescent-labeling reagent of protein or a peptide as a fluorescence compound, an indicator is preferably carried out with a fluorescein compound. For example, when carrying out an indicator with a fluorescein compound, it is following type: [0012]. Halogenation acetamide fluorescein [** 1]



[-- X expresses a halogen atom among a formula.]
[0013] A fluorescein isothiocyanate, [Formula 2]



[0014] Or eosine isothiocyanate, [Formula 3]



It is desirable to come out, to make the compound and peptide which are expressed react, and to carry out an indicator. Although it is made to combine with the cysteine in a peptide (C) when using a halogenation acetamide fluorescein as a fluorescein compound, as for the location of a cysteine, it is desirable that it is an amino terminal. When a cysteine is made to add to the amino terminal side of a peptide when the amino terminal of a peptide is not a cysteine, or a cysteine or a cystine exists in a peptide chain, the peptide chain concerned is cut in the location where a cysteine becomes an amino terminal. Moreover, when using a fluorescein isothiocyanate and eosine isothiocyanate, it is desirable to make it combine with the amino group of the amino group of the amino terminal of a peptide, lysin, or an arginine.

[0015] The peptide and the above-mentioned fluorescence compound which carry out amino acid sequence analysis are made to react, and a fluorescence labeling peptide is obtained. Fluorescence labeling peptide C (floor line) DYEGRLI (floor line: fluorescein compound) obtained at the above-mentioned reaction is refined by the usual approaches, such as high performance chromatography, and a pure article is obtained. The structure expression of CDYEGRLI which carried out fluorescence labeling with the fluorescein compound is shown in drawing 2 . Also about other peptides, a fluorescence labeling peptide can be obtained by the approach usually used by this technical field.

[0016] Next, analysis by the mass spectrometry of a fluorescence labeling peptide derivative is performed. Using the PSD technique of MALDI-TOF-MS spectroscopy, ion is excited by laser, obtained fluorescence labeling peptide derivative C (floor line) DYEGRLI is fractionation[a fragment and]-ized, and the fragment ion is detected. Thus, much fragment ion is obtained in the peptide which labeled with the fluorescence compound.

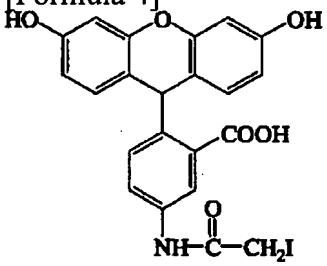
[0017] the inside am and bn (m, the number of n< amino acid residue) of the fragment (Fragmentation) pattern of a peptide as shows the fragment ion obtained as a result of the above-mentioned measurement to drawing 3 -- and -- and the peak of a large number equivalent to the mass number of the ion from which the water (H2O) molecule was removed is shown. The mass number of each fragment ion, such as the above am and bn which can be read in these peaks, is compared with the mass number of each amino acid, and the amino-acid-residue array of the peptide concerned can be determined and identified.

[0018]

[Example] Although an example is given to below about this invention and being further explained to a detail, this invention is not limited to these examples.

(Peptide sample) The nucleoprotein of the virus which has invaded by intracellular is disassembled and a nucleus peptide fragment is generated. A part of this nucleus peptide is specifically combined with antigenic proteins by intracellular. In this example, since the peptide CDYEGRLI which consists of

eight amino acid residue which is one of such the fragment peptides is very a minute amount and contained the impurity in extracting and dissociating from a cell, the sample which carried out chemosynthesis was used for measurement for establishing the new analysis method of a peptide.
[0019] (Chemosynthesis of a peptide) Chemosynthesis of the peptide of the above-mentioned array was performed with the solid phase synthesis method using the Shimadzu PSSM-8 composition machine. Removal of the protective group of synthetic compounds was performed by the standard approach currently performed from the former. High performance chromatography refined the reactant. The peptide of about 15mg pure article was obtained by one composition (98% of purity). Identification of a peptide sample was performed by checking existence of a parent ion peak (m/z:970) using the Shimadzu MALDI IV mass spectrograph.
(Labeling of a peptide) the cysteine (C) end of the above-mentioned peptide -- a fluorescence labeled compound 4(5)-(iodation acetamide) fluorescein [4(5)-(Iodoacetamide)Fluorescein]
[0020]
[Formula 4]



It came out and labeled.
[0021] The reaction condition etc. is as follows. Peptide CDYEGRLI2.8mg and 4(5)-(iodation acetamide) fluorescein 1mg were dissolved in water and 8ml (pH:7.5) of phosphoric-acid buffer solution of an acetonitrile (1:2), and it stirred for 40 minutes at the room temperature. Purification of a rough resultant was performed using Shimadzu high-performance-chromatography equipment. The elution solution used the 0.01N hydrochloric-acid water solution an acetonitrile and 80% 20%. Although two peaks were acquired in addition to the peak of the peptide of a raw material, main peaks were the peptide labeled compounds made into the purpose which the fluorescein added. About 70% of yield. The solution of this separation fraction was freeze-dried and the pure article sample was obtained.
[0022] (Analysis by the mass-spectrometry MALDI-TOF-MS method of an indicator peptide) The indicator peptide sample obtained in the experiment mentioned above was measured with the Shimadzu Kompact MALDI IV spectroscope. The parent ion peak (m/z:1356) of the whole molecule was acquired first, and it checked that the fluorescein had added. PSD of the amino acid residue of an indicator peptide -- measurement by law was performed under the following conditions, and it succeeded in obtaining SU ** KUTORU containing a good fragment ion signal. The obtained spectrum is shown in drawing 4 .
Equipment Kompact MALDI IV with :curved field reflection method Spectroscope Measuring condition : Nitrogen laser (337nm), reflectron mode Cation detection and acceleration potential are 20kV or 8kV matrix. : CHCA (alpha-cyano-4-hydroxycinnamic acid)
Sample plate conditions: Drop 0.5microL of the acetonitrile water solution which added and melted the peptide and the matrix on the plate, make it dry completely, and measure.
[0023] (Analysis of a TOF-MS-PSD spectrum) By the spectrum (drawing 4) obtained by the above-mentioned Measuring condition, main peaks, such as a3, a4, a7, b2, b3, b4, b5, b6-17, and b6, were detected in addition to the parent ion peak (m/z:1356) or the fluorescein (floor line) (refer to drawing 3 for the format of fragmentation). By carrying out comparison examination of the mass number of these peaks, the amino-acid-residue array CDYEGRLI of a peptide has been identified.
[0024]
[Effect of the Invention] When the peptide derivative which labeled the peptide with the fluorescence reagent was prepared and the sample was measured by this invention using the PSD technique of

MALDI-TOF-MS mass spectrograph, array analysis of the amino acid residue of a peptide came to be completely performed with sufficient sensibility. It is useful to structural analysis of a peptide and protein, and the analysis technique of the Alzheimer protein or cause-of-a-disease protein like a peptide attracts attention as a proteome technique of a post-genome analysis technique, and the approach of this invention has very large especially effectiveness as an important technical result of this field recently.

[Translation done.]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] The PSD spectrum of the MALDI-TOF-MS method of the nucleus peptide CDYEGRLI made by disassembling intracellular nucleoprotein.
[Drawing 2] The structure expression of the fluorescein combined with the nucleus peptide CDYEGRLI.

[Drawing 3] The cutting plane and its nomenclature of the common peptide by the PSD technique of the MALDI-TOF-MS method.
[Drawing 4] The MALDI-TOF-MS-PSD spectrum of the peptide CDYEGRLI labeled by the fluorescein.

[Translation done.]